Phenotyping Differentially Abundant Proteins in Cryopreserved vs Fresh Bull Sperm

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Semen cryopreservation is increasingly utilized in the livestock industry to preserve and distribute genetics from high indexing genetic sires. This technology has provided the ability to share high value genetics around the world and decrease biosecurity concerns involved with transporting live animals. It is not well understood, though, how cryopreservation affects the quality of preserved cells, but it is known that quality markers such as motility and membrane viability are decreased upon thawing. Mass spectrometry (MS) analysis was employed to identify distinct proteomic differences between fresh and cryopreserved sperm cells. Extracts were submitted for MS analysis at the Iowa State University Proteomics Core. A total of 2411 proteins were identified between both treatments. Of those, 402 were unique to the fresh replicates and 57 were unique to cryopreserved replicates. Principle component and cluster analysis revealed 154 proteins with differential abundances ($p<0.05$). In regard to those, 106 proteins were less abundant in cryopreserved samples and only 48 more abundant. Based on these identified proteins, three were selected for further phenotyping. The three proteins selected were cytochrome c, which was more abundant in the cryopreserved samples, 40s ribosomal protein S3a (RPS3A) and dolichyl-diphosphooligosaccharide-protein glycotransferase (DDOST), which were less abundant in cryopreserved samples. Immunocytochemistry was performed to identify protein localization within formaldehyde fixed, fresh bull sperm cells. Samples were permeabilized with 0.1% Triton X-100 and incubated with anti-cytochrome-C, anti-RPS3A, or anti-DDOST overnight before incubating with a Tritc conjugated secondary antibody, nucleotide stain Hoechst H33342 to identify the sperm nucleus, and Alexa Fluor-488 conjugated lectin PNA to observe the acrosome. Cells were mounted on ploy-L-Lysine slides and images were acquired with a Leica DM6 THUNDER upright microscope. Localization was seen in the equatorial segment by cytochrome C, RPS3A, and DDOST. Additional localization was seen in the apical ridge by labeling with PRS3A and DDOST and unique labeling in the principal piece of some cells was seen by cytochrome-c. Future steps involve elucidating localization differences between these three proteins in fresh and cryopreserved bull sperm by additional immunocytochemistry and validation of differential abundances by western blot analysis. This research aims to identify protein targets for optimization of cryoprotectants to maintain optimum cell quality and thereby ensure the reproductive potential of cryopreserved semen used in artificial insemination.

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